

Summary

Pre-mRNA splicing is a key step in gene expression, which occurs in the cell nucleus. It is accomplished by a huge complex called the spliceosome. Within the spliceosome five major snRNPs U1, U2, U4, U5, U6 and additional splicing factors undergo dynamic interactions and rearrangements to define and excise the intronic sequences from pre-mRNA to generate translatable mRNA. The whole splicing machinery is localized to the cell nucleus and some steps in snRNPs maturation occur also in the cytoplasm. snRNPs continuously cycle through nucleoplasm and nuclear compartments, like CBs and speckles to fulfill their functions. In CBs snRNPs obtain the necessary posttranscriptional modifications (pseudouridylation, 2'-O-ribose methylation) and some get pre-assembled to U4/U6 di-snRNP and U4/U6 tri-snRNP.

Pre-mRNA splicing occurs in an ordered association and dissociation of snRNPs with pre-mRNA. In the nucleoplasm the U1 and U2 snRNPs scan and recognize the intronic sequences. I showed that U1 snRNP association with 5' splice site is characteristic for a rapid exchange rate of 0.5 s. After the 3' splice site is synthesized U2 snRNP comes and this way the intron is defined. By engagement of U4/U6.U5 tri-snRNP the catalytic spliceosomal core U2.U5.U6 is generated. I showed that compared to the dynamic and fast intron recognition, the splicing reaction is the rate limiting step, which is accomplished within 30 seconds before the spliceosome disassembles.

The disassembled snRNPs are recycled and participate in the splicing process again. We showed that repeated formation of U4/U6.U5 tri-snRNP occurs mainly in Cajal bodies, where the free U5 snRNP joins the pre-assembled U4/U6 di-snRNP. This process is very dynamic and encounters productive interaction between U5 and U4/U6 snRNP specific proteins, hPrp6 and hPrp31 respectively. Mouse knockout of hPrp31 is lethal and RNAi leads to impaired formation of tri-snRNP and enlarged CBs.

Several mutations within hPrp31 protein were linked to cell specific disease retinitis pigmentosa, which affects rod cells in retina and ultimately leads to total blindness. I showed that Ala216Pro mutation in hPrp31 (AD29) impairs integration of this mutant form to U4/U6 di-snRNP, but strengthens its interaction with hPrp6 protein. Deeper study of AD29 interactions with U4/U6.U5 tri-snRNP proteins and snRNAs might bring more insights to the molecular mechanisms initiating this disease.

RB-FRET approach, which I introduced would be in this case an ideal tool for analysing RNA-protein interactions in living cells.